Microarray of Three-dimensional Heteropolymer Microstructures and Method Therefor

Claim to Domestic Priority

[0001]   The present non-provisional patent application claims priority to provisional application serial no. 60/569,370, entitled "Light Directed Solid Phase Synthesis on Patterned Polymers", filed on May 6, 2004, and further claims priority to provisional application serial no. 60/623,181, entitled "Peptide Characterized for Patterned Photopolymer Formed Using Light Directed Synthesis", filed on October 29, 2004, and further claims priority to provisional application serial no. 60/608,774, entitled "Light Activated Moving Polymers", filed on September 10, 2004.

Field of the Invention

[0002]   The present invention relates in general to arrays of chemically reactive structures and, more particularly, to a microarray of three-dimensional microstructures where the microstructures have a porous surface for providing a higher concentration of reactive sites for the patterned synthesis or attachment of functional molecular species such as heteropolymers.

Background of the Invention

[0003]   Microarrays are commonly used in the analysis of an analyte, a mixture of analytes, or some unknown compound or substance, for the purposes of identification and quantification as well as to characterize physical and

chemical properties. Microarrays can be used to determine the chemical composition, molecular structure, and properties of the analyte(s). For example, microarrays are often used to determine the presence of a specific compound or, in the case of DNA arrays, the microarray can be used to identify the presence or amount of specific gene transcripts or other specific nucleic acid sequences.

[0004] Microarrays are typically fabricated on a substrate which may consist, for example, of a silanized glass surface. Reactive chemicals or materials are disposed on the substrate in a monolayer at a number of different sites by some patterned chemical or physical process, such as photolithography. Microarray features are typically less than 10 mm, usually on order of 100 µm. Each monolayer element in the array has known reactive properties designed to bond or combine with a specific target chemical or molecular structure. Each reactive monolayer element can be selected or designed to interact with a specific target analyte. The interaction or reaction facilitates molecular recognition of the analyte. When exposed to various analytes, the reactive materials in the array elements bond or combine with the target analyte, which chemically modifies the microarray. The microarray can then be studied with analysis tools to see which element(s) reacted and thereby ascertain the composition or presence of the analyte(s).

[0005] In the prior art, microarrays are often constructed through sequential positioning of specific deprotections, and removing the protective groups from the reactive sites, followed by subsequent modification with chemical groups. Microarrays have used reactive sites with photolabile protective groups such as 6-nitroveratryloxycarbonyl (NVOC) to synthesize arrays of peptides on a glass substrate. In other microarrays, the reactive sites are protected with

photolabile groups such as (alpha-methyl-o-
nitropiperonyl)oxy]carbonyl) (MeNPOC) to synthesize DNA
arrays on glass substrates. Still other microarrays are
constructed by spotting materials of interest in specific
positions on reactive silanized glass.

[0006]   Another known characterization technique used in
DNA arrays involves the hybridization of fluorescent probes
and use of a scanning epifluorescent microscope or a
sensitive camera system to detect such probes. A
fluorescently labeled complimentary strand can be made for
each array element making it possible to characterize any DNA
microarray under the appropriate hybridization conditions.

[0007]   In any case, the density of reactive sites on the
monolayer surface of the microarray is very low, e.g. in the
range of 10-30 picomoles/cm^2. The signals from such
microarrays, which are typically fluorescent, are weak and
require sensitive detection equipment. The low signal
strength attributed to the low concentration of reactive
sites on the monolayer surface of the microarray makes
detection and analysis of the analyte difficult, and may
require use of sophisticated and expensive equipment.

Summary of the Invention

[0008]   In one embodiment, the present invention is an
array of chemically reactive sites comprising a substrate and
a plurality of three-dimensional microstructures formed on
the substrate. Each three-dimensional microstructure is made
with polymer material and has a plurality of reactive sites
formed on a surface of the three-dimensional microstructure.

[0009]   In another embodiment, the present invention is an
array of chemically reactive sites comprising a substrate and
a plurality of microstructures formed on the substrate. Each

microstructure is made with porous polymer material and has a plurality of reactive sites formed on a surface of the microstructure.

[0010]   In another embodiment, the present invention is a method of making an array of chemically reactive sites comprising providing a substrate, and disposing a plurality of three-dimensional microstructures on the substrate, each three-dimensional microstructure being made with polymer material and having plurality of reactive sites formed on a surface of the three-dimensional microstructure.

[0011]   In another embodiment, the present invention is a plurality of polymer microstructures formed on a surface of an array of chemically reactive sites. Each microstructure comprises a plurality of reactive sites disposed on a plurality of surfaces of each polymer microstructure, and each reactive site has a reactant molecule with at least one monomer.

Brief Description of the Drawings

[0012]   FIG. 1 illustrates a microarray of three-dimensional heteropolymer microstructures formed on a substrate;

FIG. 2 illustrates a microarray of heteropolymer microstructures formed on a substrate with an offset layout;

FIG. 3 illustrates a microarray of heteropolymer microstructures surrounded by other functional structures;

FIGs. 4a-4e illustrate embodiments for the three-dimensional microstructures with attached heteropolymers;

FIG. 5 illustrates a microarray with conical-shaped heteropolymer microstructures;

FIG. 6 illustrates a porous surface of the heteropolymer microstructure;

FIG. 7 illustrates another porous surface of the heteropolymer microstructure;

FIGs. 8a-8f illustrate a process of forming the heteropolymer microstructure with reactant molecules;

FIGs. 9a-9d illustrate the process of analyzing an analyte using the microarray;

FIG. 10 illustrates information from analysis of a microarray where the analysis tool is fluorescent imaging;

FIG. 11 illustrates information from analysis of a microarray where the analysis tool is mass spectrometry;

FIG. 12 illustrates information from analysis of a microarray where the analysis tool is mass spectrometry;

FIGs. 13a-13b illustrates movement of a polymer microstructure as a result of changes in swelling due to asymmetric changes in surface properties; and

FIG. 14 illustrates displacement of solvent from a polymer microstructure as seen by the movement of particles away from the polymer microstructure.

Detailed Description of the Drawings

[0013]   The present invention is described in one or more embodiments in the following description with reference to the Figures, in which like numerals represent the same or similar elements. While the invention is described in terms of the best mode for achieving the invention's objectives, it will be appreciated by those skilled in the art that it is intended to cover alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims and their equivalents as supported by the following disclosure and drawings.

[0014]   Referring to FIG. 1, a microarray 10 is shown
suitable for use in molecular detection and analysis. The
microarray is a small array of chemically reactive sites
suitable for detection of analyte(s) in any commercial
application. Microarray 10 has a substrate 12 made of
silica, glass, plastic, semiconductor materials, conducting
materials, insulating materials, arrays of semiconducting or
conducting materials such as arrays of electrodes, arrays of
functional structures such as electrodes, semiconductor
electronic components, or microcantilevers, or other suitable
physical structure support material. A plurality of polymer
microstructures 14 are formed or disposed on substrate 12.
Any two or more individual microstructures may or may not
have identical composition. In one embodiment,
microstructures 14 are made with polymer gel or porous
polymer gel or porous polymer.

[0015]   In FIG. 1, the microstructures 14 are formed in an
N x M matrix or array of identical symmetrical rows and
columns, where N and M are integers. Each microstructure 14
is a three-dimensional form having length, width, and height
or depth, as discussed further below, and designed to have
one or more reactive sites capable of bonding or combining
with one or more predetermined chemical groups, or known or
unknown analytes, i.e. each microstructure bonds with a
specific chemical, a class of chemicals, or a specific
molecular structure.

[0016]·   In general, each microstructure 14 can be
selectively tailored to be chemically reactive with one or
more specific chemical or molecular structures. The
microstructures can be designed to selectively attach
reactant molecules, such as peptides and fluorescent dye
molecules, to the microstructures by using photolabile
protective groups, or other protective groups whose chemistry

is adaptable to patterned release (electrochemical processes, for example), as discussed below. Given the tailored reactive properties of each microstructure 14, the microarray reacts when exposed to an analyte. Upon exposure, the analyte may bond, combine, or otherwise interact with the reactive site(s), which are designed to react with the given analyte or mixture of analytes. The mixture of analytes can be used to determine which heteropolymer microstructure interacts with a given analyte, or to identify analytes in the mixture which interact in some way with the heteropolymer microstructures. With the analyte bonded to or having interacted with the associated reactive site, the microarray is then studied with various analytical tools, such as mass spectrometry or fluorescent imaging, to see which site(s) have reacted with the analyte and/or to determine the identity or properties of the analyte(s) and/or microstructure(s).

[0017] The reacted sites are sometimes visible with one or more colors or intensities of light. The site(s) that have reacted can be used to indicate the identity, composition, and/or properties of the analyte(s) or microarray, a combination of the two, or to determine how the analyte has interacted with the microarray. Microarray 10 can thus be used for molecular recognition to identify the analyte and its physical and chemical properties and characteristics, properties of the array, and other properties that can be determined from the interaction of the site(s) with the analyte(s).

[0018] Substrate 12 of microarray 10 can have side on side dimensions ranging from about 1 micrometers (μm) by 1 μm to 100 millimeters (mm) by 100 mm. In the embodiment of FIG. 1, microarray 10 is shown with an array of 9 by 6 polymer microstructures 14 each having dimensions of 5 mm long, 5 mm

wide, and 150 nanometers (nm) high. The microarray can be
inset from the edge of the substrate by about 25 mm or more.
The spacing between microstructures is typically between
about 10 nm and 10 mm. In general, the density of
microstructures 14 can range up to one billion
structures/cm^2, using patterning methods known in the art.

[0019]   Turning to FIG. 2, a microarray 20 is shown with
substrate 22 having similar dimensions as microarray 10.
Microarray 20 is formed with a plurality of polymer
microstructures 24 formed in offset rows and columns, i.e.
checkerboard pattern. As discussed in FIG. 1, each
microstructure 24 is a three-dimensional form having length,
width, and height or depth, and designed to have one or more
reactive sites capable of bonding or combining with one or
more known or unknown analytes, i.e. each microstructure
interacts with one or more chemical or molecular structures.
Microarray 20 illustrates an alternate layout of the
microstructures as compared to FIG. 1. In other embodiments,
the microstructures can be formed and laid out on the
substrate in any convenient pattern useful in the molecular
recognition phase of the analysis.

[0020]   A microarray 30 is shown in FIG. 3 with substrate
32 having similar dimensions as the substrate in microarray
10. Microarray 30 is formed with a plurality of polymer
microstructures 34 disposed in an N x M matrix or array of
identical symmetrical rows and columns. Again, each
microstructure 34 is a three-dimensional form having length,
width, and height or depth, and designed to have one or more
reactive sites capable of bonding or combining with one or
more known or unknown analytes, i.e. each microstructure
interacts with a chemical or molecular structure. In this
case, the polymer microstructures 34 are surrounded by
circular functional structures 36 which have dimensions

ranging from about 100 nm to 25 mm in internal diameter. The functional structures may have any structure that adds functionality to the microarray. For example, the functional structures may serve to contain the analyte in the vicinity of the microstructures for chemical interaction. The functional structures may also operate as microfluidic channels to deliver reagents or analytes.

[0021]   FIGs. 4a-4e illustrate several embodiments of the three-dimensional geometric form and shape of the microstructures as disposed on the substrate of the microarray. The geometric shape of the microstructure is made by design choice. In general, the microstructures are three-dimensional in form having length, width, and height or depth. The three-dimensional nature of the microstructures provides additional surface area upon which to form a higher concentration of reactant molecules as compared to prior art microarray reactive sites. The higher concentration of reactant molecules increases the visual or instrumentally detected indicators of reacted sites, i.e. those microstructures to which the incident analytes have bonded or interacted. The higher concentration of reactant molecules will cause these sites to be easier to identify, read, quantify and characterize, as compared to two-dimensional monolayer arrays. The higher concentration of reactant molecules enables and facilitates the use of many analytical methods to probe the array. In the case of optical approaches, they will emit a higher intensity of light in a fluorescent assay, result in greater signal in a Raleigh or Raman scattering measurement, and provide greater absorbance for an absorbance assay. In addition, there can be greater contrast between reacted sites and adjacent non-reacted sites or for reactant sites with a different composition. The analysis of the reacted microarray is easier to perform and

may even be done with the naked eye in the case of changes in fluorescence, absorbency, or scattering in the visible region upon binding. The polymer microstructures may contain polymers or other materials which add additional properties, such as electrical conductivity, fluorescent properties, photoresponsive properties, thermally responsive properties, catalytic properties, magnetic properties, and ion conducting properties.

[0022]   In FIG. 4a, a substantially rectangular solid or parallelepiped polymer microstructure 40 is shown with an x-dimension ranging from about 10 nm to 10 mm, y-dimension ranging from about 10 nm to 10 mm, and z-dimension ranging from about 1 nm to 10 mm. The microstructure 40 can be constructed by a variety of methods including molding or casting, thermal patterning, spotting or printing, utilization of surface forces, electromagnetic patterning, patterning using selective reactivity, using radiation, using ion or molecular beams, micromachining, etching, electrochemical deposition, electrochemical reaction, chemical deposition, various types of light directed patterning, such as photolithography, laser patterning, patterned projections from liquid crystal displays or micromirror arrays, and the like. In the case of thermal, electrochemical or light directed patterning to form the microstructure, an initiator or photoinitiator can be utilized. General classes of useful photoinitiators include azides (azobisisobutyronitrile and derivatives), ketones (benzophenone), thioxanthone, acridone aromatic diketones and derivatives, ketocoumarins and coumarins derivatives, dyes (e.g. xanthene dyes such as eosin (EO) or Rose Bengal (RB), thioxanthene dyes or cyanins), thioxanthones; bis-acylphosphine oxides, peresters, pyrylium and thiopyrylium salts in the presence of additives such as a perester,

cationic dyes containing a borate anion, dyes/bis-imidazole derivatives/thiols, PS/chlorotriazine/additives, metallocene derivatives (such as titanocenes), dyes or ketones/metallocene derivatives/amines, cyanine dyes in the presence of additives, dyes/bis-imidazoles, miscellaneous systems such as phenoxazones, quinolinones, phthalocyanines, squaraines, squarylium containing azulenes, novel fluorone visible light PIs, benzopyranones, rhodamines, riboflavines, RB peroxybenzoate, PISs with good photosensitivity to the near IR, camphorquinone/peroxides, pyrromethane dye, crystal violet/benzofuranone derivatives, and two color sensitive systems. Colored cationic photoinitiators, such as iron arene salts, novel aromatic sulfonium or iodonium salts, and PS/cationic PI, where PS can be hydrocarbons or ketones or metal complexes, can help to shift the absorption in the visible wavelength range. Excited state processes of photosensitive systems for laser beams and/or conventional light sources can induce the polymerization reactions. Photosensitive systems under visible lights can include one-component systems such as bis-acylphosphine oxides, iron arene salts, peresters, organic borates, titanocenes, iminosulfonates, and oxime esters; two-component systems using through electron transfer and/or proton transfer, energy transfer, photoinduced bond cleavage via electron transfer reaction, and electron transfer; and three-component systems which enhance the photosensitivity by a combination of several components.

[0023]   Polymer or monomer systems used to form the microstructures can include monomers which are polymerized or polymers that are crosslinked or both, such as acrylate, carbonate, methacrylate, propylene, ethylene, styrene, amide, ethers (acetal), halogenated monomers, urethane, epoxy, urea, amino acids, sugars, cellulose monomers, protein, glycols,

lactic acid, ε-caprolactone, esters, nucleic acids (including DNA and RNA), peptides, trimethylene carbonate, N-vinylpyrrolidinone, and conducting polymers such as polypyrrole. The polymers/monomers can themselves contain pendent reactive groups like hydroxyl, epoxy, amino, carboxylate, vinyl, acrylate, methacrylate, or they can be incorporated after the polymerization reaction, for example amination of polyethylene.

[0024]   Light sources for polymerization can include single or multiphoton excitation using lasers, semiconductor emitters (light emitting diodes, laser diodes) or lamps. The light may be modulated using shutters, masks, movable mirrors, acousto-optic devices, Kerr-cell devices, light emitting materials (conducting polymers, dyes, semiconductor materials), liquid crystal displays, micromirror arrays and optical gating devices such as nonlinear crystals. For example, polymer microstructure 40 can be made by immobilizing a high site density material within a photopolymer matrix. In this case, a porous polymer resin with high site density is prepared by combining 0.3g 2-aminoethyl methacrylate, 1.95 g poly(ethylene glycol)dimethacrylate, 1.39g trimethylolpropane ethoxylate (14/3 EO/OH) triacrylate, 50 mg azo-bis-isobutyronitrile, and 8 ml cyclohexanol. Nitrogen is bubbled through the solution for 10 minutes to remove oxygen and then the solution is heated to approximately 90°C for approximately 20 minutes. After polymerization the polymer is ground in a mortar and pestle, washed with a pH 2 TFA water solution, water, methanol, dried, and dry sieved with a 75 micron (μm) sieve. About 20 milligrams (mg) of the above resin is swollen in 40 microliters (μL) methanol and suspended in a 1% 2,2'-azobisisobutyronitrile (AIBN) in trimethylolpropane trimethacrylate (TRIM) solution. Nitrogen is bubbled through

the solution for 10 minutes to remove oxygen before loading
it into a nitrogen purged flow cell with the functionalized
glass slide and a 250 μm thick gasket separating the
coverslip from the upper glass slide. The resin is
polymerized using a micromirror array with a 380/50nm
bandpass filter for 5 minutes at an intensity of 54mW/cm^2.
The resultant shape is 250 μm cubes, which are rinsed with
methanol and N,N'-dimethylformamide to remove unpolymerized
monomer and the gasket is replaced with a 600 micron gasket
to facilitate mixing. This example demonstrates the
construction and formulation of polymer microstructures using
a lamp and micromirror array where the microstructure is
comprised of a polymer giving the microstructure shape and
function and a high site density material which gives the
polymer microstructures high densities of reactive sites.

[0025]   In FIG. 4b, a substantially cylindrical polymer
microstructure 42 is shown with a diameter ranging from about
10 nm to 10 mm and z-dimension ranging from about 1 nm to 10
mm. In one embodiment, microstructure 42 can be constructed
using similar methods as described in FIG. 4a, wherein the
photopolymer gel structures are constructed directly from a
material bearing reactive sites. Microstructures are
prepared from a deoxygenated solution of 166 mg 2-amino ethyl
methacrylate, 805 mg trimethylolpropane ethoxylate (14/3
EO/OH) triacrylate, 9.7 mg azo-bis-isobutyronitrile, 1188 mg
cyclohexanol (note other solvents can be used to increase
pore size for example mixtures of 1-dodecanol and
cyclohexanol) in an optical cell with 250 μm gasket and 3-
(trimethoxysilyl)propyl methacrylate functionalized glass
slide as described in FIG. 8a. The polymerization is done
with excitation from a micromirror array using a 380/50
excitation filter and exposing the features for 13 minutes.
The structures are washed with methanol for more than 12

hours. One spot is tested with a 1% solution of 2,4,6-
trinitorbenzenesulfonic acid (TNBS) in DMF which turns bright
orange indicating the presence of primary amines. The
resulting structure shows the direct construction of polymer
microstructures from monomers one or more of which contains a
reactive site that is incorporated into the polymer
microstructures.

[0026]   In FIG. 4c, a substantially conical polymer
microstructure 44 is shown having base diameters ranging from
about 10 nm to 10 mm and z-dimensions ranging from about 1 nm
to 10 mm. The microstructure 44 can be constructed using
similar methods as described in FIG. 4a. In one embodiment,
a scanning laser system has been used to generate three-
dimensional TRIM crosslinked poly(2-hydroxylethyl
methacrylate) (HEMA) polymer microstructures through azo-bis-
isobutyronitrile (AIBN) photopolymerization using a 20x 0.5NA
microscope objective and 365 nm laser excitation. A solution
containing as solution of TRIM, HEMA, AIBN for example
approximately 773 μl TRIM, 128 μL HEMA, 9 mg AIBN. The AIBN
is prepared without removing oxygen and placed in the optical
chamber, containing a silanized slide prepared as described
in FIG. 8a, and irradiated at room temperature with 2-4 mW of
365 nm light for 1-2 seconds per feature. A 10x or 20x
objective is typically used to focus the light on order of
100 μm above the surface of the cover slip to construct
microarrays with features spaced on order of 500 μm apart.
In general taller features are made with more laser power
and/or longer exposures with the focus further about the
surface. Excess monomer is drained and sample washed with
methanol and DMF. These microstructures are later modified
as described in FIG. 8c. Large pores are formed in regions
of highest light flux. The resulting structure demonstrates
the construction of polymer microstructures using a laser

system where the microstructures made using a nonlinear
process and are modified after polymerization to change their
reactivity, which also demonstrates the three dimensional
control of pores formation through the use of control of
light intensity.

[0027]   In FIG. 4d, a substantially cylindrical polymer
microstructure 46 is formed in an inverted manner as a well
or depression. The three-dimensional well-shaped
microstructure 46 has a diameter ranging from about 10 nm to
10 mm, and z-dimension ranging from about 1 nm to 10 mm. The
microstructure 46 can be constructed using similar methods as
described in FIG. 4a. In one embodiment, the well-shaped
microstructures can be made by placing several microliters of
a monomer solution containing a free radical initiator in
Teflon printed slides. The monomer is made from 10
microliters (µL) glycidyl methacrylate, 90 trimethylolpropane
trimethacrylate solution containing 1% by AIBN. The 20 µL of
the solution is placed in a 4 well Teflon printed slide, 5 mm
well diameter, and placed in an oxygen free heated chamber at
85°C for 1 hour. The structures can be later modified to
modulate their reactivity, e.g. by reacting with a 10%
solution of 1,4-Bis(3-aminopropoxy)butane in DMF for 15
minutes modifies the reactivity of the polymer
microstructures and results in cylindrical microstructures
with domed tops. The resulting structure demonstrates the
use of surface forces to pattern polymer microstructures and
post polymerization modification of the microstructures to
change the reactive sites.

[0028]   In FIG. 4e, an irregular-shaped polymer
microstructure 48 has x-dimensions ranging from about 10 nm
to 10 mm, y-dimensions ranging from about 10 nm to 10 mm, and
z-dimensions ranging from 1 nm to 5 mm. The microstructure

48 can be constructed using similar methods as described in
FIG. 4a.

[0029]   Turning to FIG. 5, a portion of an array of polymer
microstructures is shown with conical microstructures like 44
from FIG. 4c formed on substrate 50. Arrays of
microstructures can be made using any of the methods
described in FIG. 4a-4e. The spacing between microstructures
can be in the range of about 10 nm to 10 mm. Typical
concentrations of reactive sites as determined using methods
described in FIG. 8c are found to be on order of 0.1
nmole/feature for features on the order of 100 microns in all
dimensions, providing an apparent surface density of on the
order of 0.01 micromoles/cm^2 which represents a 10,000 fold
enhancement over the prior art.

[0030]   Notice that the microstructures are made three-
dimensional in shape to achieve greater reactive surface
area. The three-dimensional nature of the microstructures
provides additional surface area upon which to form a higher
concentration of reactant molecules. The higher
concentration of reactant molecules per microarray reactive
site makes it easier to determine the properties of the
microarray and molecules bound to it and allows the use of
analysis tools that are currently not commonly used for
microarrays. For example, the higher concentration of
reactant molecules will cause the reacted sites to emit
higher intensity of light when interacted with a fluorescent
material and can also have a greater contrast to adjacent
non-reacted sites. Also, sites with such high concentrations
of reactive groups allow the use of other analytical tools
such as mass spectrometry.

[0031]   Referring now to FIG. 6, a portion of polymer
microstructure 44 is shown with porous regions 54 which are
on order of a micrometer in feature size. The microstructure

44 can be made with polymer gels with porous structures such
as regions 54 or rigid porous structures, or of nonporous
polymers and polymer gels, which can be made by swelling in
solvent, partial polymerization, partial cross-linking, phase
separation, use of emulsions, trapping or formation of gas
bubbles, polymerization of monomers containing a suspension
of pore templates which are later dissolved, suspensions of
porous materials, and post polymerization modification. The
porous regions 54 may form without the use of porogens in
regions of highest light flux due to phase separation during
polymerization. The microstructures 44, like most polymer
structures, will swell in a compatible solvent facilitating
diffusion of reactants or analytes and increasing the
available surface area versus the external surface area by
several orders of magnitude.

[0032]   In other words, the porous nature of the three-
dimensional microstructures as shown in FIG. 6 further
increases surface area upon which to form a higher
concentration of reactant molecules. The higher
concentration of reactant molecules per microarray reactive
site increases the visual or instrumentally detected
indicators of reacted sites. For example, the higher
concentration of reactant molecules will cause the reacted
sites to emit higher intensity of light when binding of an
analyte results in fluorescence and with greater contrast to
adjacent non-reacted sites.

[0033]   Given the pores surrounded by polymer gel, the
pores can greatly facilitate the diffusion of materials in
and out of the polymer gel by acting as channels for
reactants or analytes. In the case of microstructures like
40 as described in FIG. 4a, pores are obtained as a result of
suspension of a porous solid phase synthesis material

therefore allowing the independent optimization of
photopolymer properties and solid phase synthesis properties.
[0034]   In the case of microstructures 42 as described in
FIG. 4b a porogen cyclohexane is used to form the porous
structures as a result of phase separation during
polymerization. Porogens can include any solvent, common
ones include cyclohexane, methanol, 1-dodecanol,
acetonitrile, ethylacetate, and polymeric porogens such as
polystyrene particles. In the case of microstructures 44 as
described in FIG. 4c, high light intensity is used to form
pores without the use of a porogen and the largest pores are
found to form in regions of highest intensity, which
demonstrates the three dimensional control of pore formation
in polymeric materials.

[0035]   In FIG. 7, a portion of polymer microstructure 42
is shown with porous regions 56 on the surface and interior
portions of the microstructure. The porous regions 56 are on
order of one micrometer in feature size. Typically, these
structures have concentrations of reactive sites as
determined by methods described in FIG. 8c on order of 10
nmoles/feature for features with dimensions on the order of
100 microns, providing an apparent surface density of on the
order of 1 micromoles/cm^2 which represents a million fold
enhancement over the prior art. Large pores speed diffusion
decreasing reaction times and solvent dependence of the
polymer. A polymer gel, even without large pores, will swell
in an organic solvent allowing the attachment of active
groups however. The same polymer gel may not swell in a
different solvent, such as water. If the analyte to be
tested requires water as the primary solvent, the analyte may
not be able to interact with the active groups restricting
the interaction to the outer surface of the microstructure.
By forming large pores or macropores, the solvent dependence

of the microstructure is greatly reduced because the reagents
and analyte can access the internal area of the polymer
through the macropores. In the case of microstructures 44, a
porogens cyclohexane or mixtures of cyclohexane and 1-
dodecanol are used to form the porous structures as a result
of phase separation during polymerization. Porogens can
include any solvent, common ones include cyclohexane,
methanol, 1-dodecanol, acetonitrile, ethylacetate, and
polymeric porogens such as polystyrene particles.

[0036]   Again, the porous nature of the three-dimensional
microstructures as shown in FIG. 7 further increases surface
area upon which to form a higher concentration of reactant
molecules. The higher concentration of reactant molecules
per microarray reactive site facilitates use and analysis,
including visual and instrument detected indicators of
reacted sites. The higher concentration of reactant
molecules will cause the reacted sites to emit a higher
intensity of fluorescence and/or with greater contrast to
adjacent non-reacted sites, and facilitating the use of
fluorescence detection or allowing the use of other
analytical tools such as mass spectrometry.

[0037]   There may be sufficient material on the polymer
microstructures to allow the use of mass spectrometry to
determine the molecular structure of materials bound or
interacting with the polymer microstructure(s). Enabling the
use of analytical tools such as mass spectrometry is
significant because they can allow the determination of
precise details of the molecular structure of materials on
the array or interacting with the array, as compared to
fluorescence or absorbance which in general gives little
detailed information other then the presence or absence of a
material.

[0038]   The presence of the analyte bound to the reactive
microstructure may change the chemical reactivity of the
system. One instance would be a change in the
electrochemical properties of the system allowing one to
perform electrochemical assays of binding, particularly if
the substrate consists of an array of electrodes.

[0039]   In another example, the presence of the analyte
might change the electrical conductance of the
microstructure, which would be of particular interest if the
microstructure is fabricated in such a way as to form a
bridge between two electrodes on the substrate. The binding
of analyte to the microstructure could change the total mass
of the microstructure and therefore change the forces
involved in accelerating the microstructure, which is
particularly important in situations in which the
microstructures are fabricated on microbalance devices such
as oscillating cantilevers, where the change in mass results
in a change in the characteristic frequency of the
cantilever.

[0040]   Alternatively, the binding of the analyte to the
microstructure could change the total mass of the
microstructure and therefore change the forces involved in
accelerating the microstructure, which is particularly
important in situations in which the microstructures are
fabricated on microbalance devices such as oscillating
cantilevers, where the change in mass results in a change in
the characteristic frequency of the cantilever.

[0041]   In yet another example, binding of the analyte to
the microstructure could result in changes in the electrical
properties of a semiconductor based electronic device (for
example, a field effect transistor or a bipolar transistor or
a diode) in a detectable fashion, which is of particular

importance if the microstructures on fabricated directly on arrays of semiconductor devices or sensing elements.

[0042]   In another example, the binding of the analyte to the microstructure could change the volume or solvation/hydration properties of the microstructure, which in turn could change the physical properties of the microstructure in a detectable way, for example, a change in index of refraction.

[0043]   FIGs. 8a-8f illustrate the steps of making the microarray with microstructures and reactant molecules. The microarray uses spatially addressable synthesis. That is, microstructures like 62 and 63 in FIG. 8b, which in general can be located anywhere on the microarray, can be made with different polymer materials and exhibit different chemical properties. Likewise, the chemical groups formed on any specific microstructure can be different. For example, FIG. 8f illustrates microstructure 62 with two chemical groups having different chemical structures, i.e. L-M1-M2-M3-M4 and L-M1-M2-M3-P. Thus, each microstructure formed on substrate 60 can be selectively made of different polymer materials and the reactant molecules on each microstructure can be made to be chemically reactive with any of a broad range of analytes.

[0044]   In FIG. 8a, a substrate 60 is made of silica, glass, or plastic, as described for 12 and has dimensions ranging from 100 mm to 1 μm in the x-dimension, 100 mm 1 μm in the y-dimension, and 1 mm to 50 μm in the z-dimension. In this case, the polymer microstructures will be covalently linked to the substrate. In the case of glass, many other oxides such as functionalized silanes provide a facile method for linking the polymer to the substrate. The glass surface will react with the silane to form a silicon oxygen bond, the other end of the silane can be selected so that it will react

with the polymer or monomer therefore providing a covalent
linkage between the polymer and the glass surface.

[0045]   The silanization of the glass substrate is
performed as follows. Glass cover slides are cleaned. The
slides are immersed for 15 minutes at room temperature with
60/40 (v/v) sulfuric acid/hydrogen peroxide, 10% sodium
hydroxide (w/v) at 70°C for 3 minutes and 1% HCl at room
temperature for 1 minute. Between steps, the slides are
soaked in nanopure water for 3 minutes. A solution of 1-5%
3-(trimethoxysilyl)propyl methacrylate or
aminopropyltriethoxysilane (APTES) in 95% ethanol/ 5 % water
is prepared and mixed for 10 minutes. The slides are
immersed in the silane solution at room temperature for 15
minutes with gentle agitation. Slides are soaked in
isopropyl alcohol for 3 minutes, nanopure water for 1 minute,
and placed in a 100°C oven for 5 minutes after which the oven
is turned off and nitrogen is blown through for 1 hour. The
slides are stored under nitrogen or argon.

[0046]   In FIG. 8b, polymer microstructures 62 and 63 are
attached to substrate 60. The microstructures 62-63 can be
spaced as described in FIG. 2 and FIG. 3. The
microstructures 62-63 can be covalently attached to an oxide
surface such as glass using methods like that described in
FIG. 8a. However, depending upon the application, it is not
always necessary to have a covalent linkage between the
microstructure and the substrate. Physisorption of the
monomer or polymer onto the substrate surface can
sufficiently immobilize of the polymer microstructures for
some applications. The interactions may be enhanced by using
a rough or etched or patterned surface thereby increase the
area of contact and the strength of the immobilization and
other functional properties.

[0047]   In FIG. 8c, a portion of polymer microstructure 62
is shown. The internal and external surface of the polymer
microstructure 62 is modified with a linker group L 64. The
linker molecule can provide a great deal of functionality.
Linking molecules are often used to change surface
properties, e.g. polyethyleneglycol linkers are commonly used
to prevent the nonspecific binding of proteins to surfaces.
Linkers can also amplify the number of sites at the surface
by coupling of dendrimeric materials such as PAMAM dendrimers
or other multifunctional groups and can be used to introduce
or increase the number of reactive sites or to modulate the
surface properties of the polymer. Examples of other types
of linking or surface modifying groups include, sugars, amino
acids, nucleic acids such as DNA and RNA, peptides, proteins,
polysaccharides, and other multifunctional amines or
alcohols.

[0048]   Linkers can also provide chemical functionality,
i.e. the linker can be selectively cleaved allowing the
removal of the group that is attached to the surface.
Examples of labile linkers include acid labile linkers such
the RINK amide linker, oxidativly labile hydrazinobenzoyl
linker, base labile and/or linkers cleaved by nucleophiles
such as 4-hydroxymethyl benzoic acid linkers, or photolabile
linkers such as the hydroxyethyl photolinker, can be used to
selectively remove materials from the polymer surface.

[0049]   In peptide synthesis, orthogonal protective groups
are used to modulate the various functional groups of the
monomers and polymers. Side chain protective groups prevent
the side chains from reacting during coupling steps. In
general, the protective groups should not be labile under the
conditions required during the removal of the protective
groups used to control the growth of the polymer chain. In
this case attached to the linker group is a protective group

P 66.   Protective groups include acid labile, base labile,
reductively or oxidatively labile, thermally labile,
electrochemically labile, and photolabile protective groups.
Common groups include acid labile 4,4'-Dimethoxytrityl (DMT)
or other tryityl derivatives, tert butyoxycarbonyl (BOC) and
tert butyl (t-but) groups, base labile groups such 9-
Fluorenylmethoxycarbonyl (FMOC), reductively labile groups
such as the benzyloxycarbonyl group (cbz), and photolabile
protecting agents such as aromatic nitro compounds such as
nitroveratryloxycarbonyl (NVOC), 5'-((α-methyl-2-
nitropiperonyloxy)carbonyl, (alpha-methyl-o-
nitropiperonyl)oxy]carbonyl (MeNPoc), 2-(2-
nitrophenyl)ethoxycabonyl, 2-(2-nitrophenyl)ethylsulfonyl,
and nitrophenylpropyloxycarbonyl.   Other groups include 1-
pyrenylmethyloxycarbonyl, alpha-Ketoester derivatives, Benzyl
alcohol derivatives, Benzoin derivatives, Phenacyl esters,
coumarin derivatives, hydroxyphenacyl, and benzyloxycarbonyl.
[0050]   During chemical synthesis, it is often desirable to
utilize a solvent that will swell microstructures to form
polymer gels and solvate the growing polymer chain and/or
reactants.   Anhydrous solvents with the appropriate solvation
properties are typically desirable given these considerations
though water is often used for certain reactions such as
attachment of DNA to reactive polymer microstructures.
Common solvents include acetonitrile, N,N-Dimethylformamide
(DMF), Dimethyl sulfoxide, 1-Methyl-2-pyrrolidone (NMP), and
tetrahydrofuran(THF).   In one embodiment, Fmoc-Rink or Fmoc-
Gly is coupled to the hydroxyl group on the photopolymer
microstructures prepared in FIG. 4c by reacting a solution of
either using 33.7 mg Fmoc-Rink or 18.6 mg Fmoc-Gly, 22.5 mg
HBTU, 11.5 μL DIPEA, and 600 μL DMF.   The Fmoc-Rink or Fmoc-
Gly, DIPEA, and DMF are combined and reacted for 3 minutes,
and then added to the chamber and reacted with mixing at 50°C

for 30 minutes.   The microstructures are rinsed with DMF and
the Fmoc removed with 20% piperidine in DMF for 10 minutes.
The yield of the reaction and number of reactive sites is
determined by monitoring the absorbance at 301 nm of the
Fmoc-piperidine adduct released.

[0051]   In FIG. 8d, a portion of polymer microstructures
62, where internal and external surface of the polymer
microstructure, is modified with a linker group L 64.   A
protective group P 66 is attached to the linker group.   The
protective group is selectively removed from the linkers in
some positions using optical, electrical, thermal, or
chemical means.   The overall yield can be increased through
the selection of appropriate deprotection conditions, such as
temperature, pH, chemical content, solvent, light intensity,
and electrochemical potential.   In many cases, it is
desirable to include scavengers when removing these
protective groups to prevent reactive cleavage products from
reacting with materials attached to the polymer
microstructures.   Examples of scavengers include triisopropyl
silane can be used to scavenge cations resulting from the
cleavage of t-but from a hydroxyl group using trifluoracetic
acid (TFA) and semicarbazide HCl can be used to scavenge the
aldehyde photocleavage product that results when
nitroveratryloxycarbonyl is cleaved from an amino group.

[0052]   In FIG. 8e is shown a portion of polymer
microstructures 62 where internal and external surface of the
polymer microstructure is modified with a linker group L 64.
A protective group P 66 is attached to the linker group.   The
protective group has been selectively removed from the
linkers is some positions using optical, electrical, thermal,
or chemical processing and a monomer M1 68 has been attached
bearing protective group P 66.   Monomers can include amino
acids, peptides, proteins, nucleotides, oligonucleotides,

DNA, RNA, sugars, polysaccharides, polyethyleneglycols, lipids and derivatives, polymers, and/or other inorganic or organic molecules.

[0053]   Consider the preparation of the polymer microstructures as described in FIG. 4a. A solution of 12 mg of the peptide FMOC-GGFL-COOH, 5.4 mg HBTU, 13 µL DIPEA, and 500 µL DMF is allowed to react for 3 minutes and then added to the microstructures. The microstructure is reacted for 1 hour at 50°C and rinsed with DMF until the solution OD301nm < 0.2. After a 10 minute 20% piperidine in DMF rinse the solution OD301 is 0.97, the microstructure is rinsed with DMF and reacted with a solution containing 19mg NVOC, 40 µL DIPEA, 600 µL DMF and reacted 1 hour in the dark at room temperature. The microstructure is rinsed with DMF and a solution of 1% (w/w) semicarbazide HCl in methanol and allowed to soak in same solution for 10 minutes. One half of the array is illuminated for 5 minutes using the same apparatus used to make the microstructures. The microstructure is rinsed with methanol and DMF and a solution of 1 mg N-hydroxysuccinimide ester of N–Tris(2,4,6-trimethoxyphenyl)phosphonium acetic acid (TMPP-Ac-OSu-Br) 20 µL DIPEA, and 480 µL DMF is added and allowed to react at 35°C for 30 minutes. The microstructure is then rinsed with DMF and methanol. The resulting structure demonstrates spatially addressable synthesis on the polymer microstructures decribed in FIG. 4a.

[0054]   In FIG. 8f shows a portion of polymer microstructures 62 where internal and external surface of the polymer microstructure is modified with a linker group L 64. A protective group P 66 is attached to the linker group. The protective groups are selectively removed from the linkers in some positions using light, electrical, thermal, or chemical

means to construct two types of polymers. One polymer contains monomers M1 68, M3 70, and M4 72. The second polymer contains monomers M2 74, M3 70, and M5 76.

[0055] Consider the preparation of the polymer microstructures as described in FIG. 4c, Fmoc synthesis is used to make Phe-Leu-Phe (FLF) on the polymer microstructures. The coupling steps are performed by reacting 63 μmoles of Fmoc-amino acid or peptide or Fmoc-Rink, 22.5 mg (59 μmoles, 0.94 eq) HBTU and 11.5 μL (66 μmoles, 1.5eq) DIPEA in 600 μL DMF for 3 minutes, adding to the microstructures and reacting at 50°C for 1 hour. The microstructure array is rinsed with DMF until the absorbance at 301 nm < 0.1, and washed twice for 10 minutes with 20% piperidine in DMF, then washed with DMF to remove the piperidine. The absorbance at 301 nm is measured and compared to that of the Fmoc cleavage following addition of the linker as described in FIG. 8c to determine the stepwise yield.

[0056] NVOC-Phe is then coupled as described above with the exception that coupling is done at room temperature in the dark overnight. The surface is acylated by adding a solution of 29.2 μL DIPEA, 20 μL acetic anhydride, 1 μL DMF and allowing it to react for 1 hour at room temperature. The microstructure is rinsed in dioxane and left in dioxane. The same laser system used to make the structures is used to selectively remove the photolabile protective group from half of the features. Fmoc-leucine is coupled and the Fmoc group removed as described above. The resulting structure demonstrates spatially directed synthesis where two types of protective groups are used, one chemically labile and the other photolabile.

[0057]   Accordingly, FIG. 8f illustrates a plurality of chemical groups respectively attached to reactive sites on the surface of the three-dimensional microstructure by the above process. One chemical group is L 64, M1 68, M2 70, M3 72 and M4 74. Another chemical group is L 64, M1 74, M2 70, M3 76, and P 78. As shown, each chemical group including at least one monomer M_i. Note that the chemical groups have different chemical structures.

[0058]   FIGs. 9a-9d illustrate the steps of exposing a microarray to an analyte, bonding the analyte to one or more reactive microstructure sites, and then analyzing the reacted microarray with analysis tools. The analysis tool provides identification and other information related to the analyte and/or microstructure.

[0059]   In FIG. 9a, a portion of a polymer microarray is shown with microstructures 82, 84, 86, 88, 90, 92, 94, and 96 attached to substrate 80. The microstructures and substrate having dimensions, properties, order, preparation, and spacing as described in the preceding figures. The analyte can be introduced to the microstructures via a variety of methods including pressure driven flow, spotting of analyte, deposition of vapors or sprays, deposition of particles, exposure to gases, direct contact of the array with surfaces, and emersion of the array in test solution. Possible analytes of interest include biological materials, inorganic materials, and organic materials. Biological materials include proteins or peptides, DNA or RNA, sugars or polysaccharides, lipids, cells, organisms, and combinations thereof. Inorganic materials include metal ions, salts, inorganic complexes, and minerals. Organic materials include, drugs, dyes, toxins, pollutants, restricted substances and surfactants.

[0060]   In FIG. 9b, the microarray from FIG. 9a is shown
after treatment with analyte. In the present case, the
analyte has interacted with the polymer microstructures 86
and 92. The interactions can include molecular recognition,
chemical modification, enzymatic modification, cellular
interactions, and catalytic properties. The molecular
recognition feature involves binding of peptides to proteins
or other materials of interest or binding of DNA to another
nucleic acid such as RNA, peptides, proteins, and antibodies.
The chemical modification feature involves oxidation or
reduction of materials, changes in oxidation or reduction
potentials, and chemical reactions. The enzymatic
modification feature involves modification of proteins
(phosphorylation, glycosylation, methylation, acetylation,
proteolytic cleavage), electrochemical activity, proteolytic
activity, nuclease activity, and other enzymatic reactions.
The cellular interactions involve changes in
biocompatibility, biofilm formation or inhibition, and
changes in cell differentiation, cell-cell interactions,
surface adhesion, cellular metabolism and proliferation. The
catalytic properties include changes in electrochemical
reaction rates, sterospecific reactions, polymer cleavage
reactions, and synthetic organic catalytic properties.

[0061]   In FIG. 9c, the microarray from FIG. 9b is shown
undergoing evaluation by analysis tool 94. The analysis tool
94 is used to characterize the polymer microarray. The
analysis tool reveals that polymer microstructures 86 and 92
have interacted with the analyte. Analysis tools include,
UV-Visible-Infrared spectroscopy, Colorimetry, fluorescence
spectroscopy, Raman spectroscopy, mass spectrometry, optical
microscopy, circular dichorism, Fourier Transform Infrared
Spectroscopy, ellipsometry, scanning probe microscopy,
cantilever resonance, surface plasmon resonance spectroscopy

electrochemical detection, electronic detection and high vacuum techniques including x-ray photoelectron spectroscopy.

[0062]   In FIG. 9d, the polymer microarray in FIG. 9b as revealed by the analysis tool in FIG. 9c. The analysis tool revealed that polymer microstructures 86 and 92 have interacted with the analyte due to a change in their spectroscopic properties. In a colorometric test, the interaction may indicate some enzymatic or chemical activity. In another example, the presence of a fluorescent group demonstrates an interaction with structures 86 and 92 and not with the other microstructures. The microarray format provides an easy method for detecting and comparing changes in many of the microstructures at once.

[0063]   In FIG. 10, the microarray is shown through the analysis tool 94 as described in FIG. 9d. In this case, the analysis tool 94 is a fluorescent imaging system. The analysis tool 94 reveals polymer microstructures 102 interacting with the analyte to show green fluorescence. Other polymer microstructures 104 have interacted with the analyte to show red fluorescence. The fluorescent groups could be any groups that can be differentiated on the basis of fluorescent properties such as the fluorescence spectrum, anisotropy or lifetime, e.g. the indicator groups could be fluorescently labeled DNA or RNA that have hybridized to the complimentary DNA or RNA on the microstructures or fluorescently labeled antibodies which are binding specifically to structures 102 and 104 due to molecular recognition. The polymer microstructures are prepared as described in FIG. 4c, Fmoc-Glycine is attached and then deprotected as described in FIG. 8c, the NVOC is attached to the microstructures and selectively removed as described in FIG. 8e with the exception that 60 mM semicarbazide with 3% DIPEA in DMF is used as the scavenging solution, the array is

washed and treated with 4 mg Fluorescein isothiocyanate
(FITC) in 600 µL DMF at 50°C for 30 min. The array is washed
and the above process is repeated to selectively remove NVOC,
the array is washed and treated with 1 mg/mL Texas Red
Sulfonyl Chloride (TR-SC) in DMF for 2 hours at room
temperature and rinsed with DMF. The analysis tool 94 is an
apparatus consisting of a Hg lamp to excite the dyes, a CCD
camera to take images, and optical filters to provide the
correct excitation and emission light. For FITC a 480/20
filter is used for excitation and a 540/25 filter is used for
emission. For TR-SC a 560/40 filter is used for excitation
and a 630/60 is used for emission. The two images are
superimposed. The FITC (green fluorescence) is in the areas
patterned first and the TR-SC (red fluorescence) is in the
areas patterned second. In this case, the fluorescence is
easily be seen by eye. The fluorescence from the polymer
microstructures reveal an increase in fluorescence signal
from the polymer microstructures on the order of 10,000-fold
over the monolayers. The resulting structure demonstrates
the selective attachment of fluorescent dyes and fluorescent
detection as an analysis tool. The resulting structure
further demonstrates spatially addressable synthesis on the
polymer microstructures and the application of an analytical
tool to determine the properties of the resulting
microstructures.

[0064]   As a second example involving the microstructures
described in FIG. 4b, the polymer microstructures are treated
with a solution of 102.5 mg N,N'-Disuccinimidyl carbonate,
66.1 µL DIPEA, and 8 mL acetonitrile for 4 hours. Single
stranded DNA at 560 micromolar concentration in tris-HCl
buffer pH 8 and 100mM NaCl containing a 5' amino group is
spotted at 1-10 µL onto half of the features and the same
procedure is used to spot a second sequence of DNA containing

a 5′ amino group and allowed to react overnight. These two
sequences are selected so that they will have specific
interactions with their fluorescently labeled complimentary
DNA probes and not with the noncomplimentary probe. The
spots are passivated using a 150 micromolar solution of 1,4-
Bis(3-aminopropoxy)butane in water for a few hours, and
washed in buffer. The complimentary strands bearing green
(FITC) and red fluorescent (Texas RedX) groups are hybridized
and the array is washed and imaged as described in the
previous example or simply using a lamp an inexpensive
consumer digital camera with the same filters. Again the
fluorescence is clearly visible by eye. The images of
fluorescent structures with the appropriately positioned red
fluorescent and green fluorescent DNA are obtained, which
demonstrates the construction and use of polymer microarrays
where the analyte interacts through molecular recognition
with the array and is detected by fluorescence.

[0065]   In FIG. 11, a microarray is revealed by the
analytical tool described in FIG. 9d, wherein the tool is a
mass spectrometer indicating that the mass 110 is 964.4 Da.
The microarray is prepared as described in FIG. 8e and the
analysis tool is Matrix Assisted Laser Desorption Ionization
Mass Spectrometry MALDI-MS. The features are photopatterned
and treated with the TMPP group, and then treated with a
trifluoroacetic acid (TFA) solution. The solution is
combined with the mass spectrometry matrices 4-
hydroxybenzylidenemalononitrile (4-OH BMN) or α-cyano-4-
hydroxycinnamic acid which are dissolved in 50% acetonitrile,
0.1% TFA, and nanopure water. The solution is assayed using
the MALDI-MS. The resulting spectrum shows the product TMPP-
GGFL-amide mass at 964.4 Da vs. 964.4 Da theoretical mass,
the three major isotopes (964.4, 965.4, and 966.4) are also
clearly visible and match the correct theoretical isotopic

distribution expected from the photopatterned
microstructures. These are not found in the unexposed
miocrostructures, which demonstrates the synthesis of a
modified peptide array using light and attachment of a
peptide to the polymer microstructures.

[0066]   In FIG. 12, a microarray is revealed by the
analytical tool described in FIG. 9d, wherein the tool is a
mass spectrometer indicating that the mass 112 is 560.3 Da.
The microarray is prepared as described in FIG. 8f and the
analysis tool is Matrix Assisted Laser Desorption Ionization
Mass Spectrometry MALDI-MS. MALDI-MS is preformed as
described in FIG. 11 resulting the detection of the LFFL-
amide Na+ ion (560.3 Da vs 560.3 Da theoretical mass) in the
arras that had been selectively patterned using light, which
demonstrates the successful synthesis of a peptide microarray
using multiple protective groups including chemical and
photolabile groups and use of mass spectrometry as an
analysis tool. The product is not found from microstructures
in the areas not exposed to light, which demonstrates the
chemically directed synthesis of a peptide on polymer
microstructures and the use of mass spectrometry as an
analysis tool.

[0067]   In FIGs 13a-13b, is illustrated the movement of a
polymer microstructure 120 as a result of changes in swelling
due to asymmetric changes in surface properties. Light
induces photochemical changes in the surface chemistry of
porous polymer microstructures giving rise to a substantial
change in volume. When illumination is asymmetric, the
structure undergoes light-directed motion.

[0068]   In FIG. 13a, the polymer microstructure 120 is
standing up before exposure to light. In this case, polymer
microstructures are prepared as described in FIG. 4c, these
swellable trimethylolpropane trimethacrylate (TRIM)

crosslinked poly(2-hydroxylethyl methacrylate)
microstructures are aminiated with glycine and protected with
the photolabile group 4-nitroveratryloxycarbanyl (NVOC) as
described in FIG. 8c and 8e. Addition of NVOC resulted in a
volume increase >10% when performed in the solvent N,N'-
dimethylformamide (DMF).

[0069]   In FIG. 13b, the polymer microstructure 120 is bent
over as a result of asymmetric exposure to light.
Photochemical cleavage of NVOC is shown using asymmetric
illumination as described in FIG. 4c of a microstructure 44
with a 365nm laser induced polymer shrinkage in excess of 4%
at the base of the cone and resulted in a maximum velocity of
1mm/s at the tip of the cone, which demonstrates the use of a
change in surface properties of the polymer microstructure to
result in the directed movement of the polymer
microstructure.

[0070]   FIG. 14 illustrates the displacement of materials
from polymer microstructure 120 as seen by the movement of
particles away from the polymer microstructure. The movement
of particles from microstructures as described in FIG. 13a-
13b is away polymer upon symmetric illumination in the center
of microstructure base with 365 nm light for 20sec at 400 µW
with 10x objective lens as described in FIG. 13b. The
particle trajectories appear as dotted lines, as a result of
changes in swelling due to sudden changes in surface
properties. Light induces photochemical changes in the
surface chemistry of porous polymer microstructures giving
rise to a substantial change in volume. Photochemical
cleavage of NVOC using illumination as described in FIG. 4c
of microstructure 44 with a 365nm laser induced polymer
shrinkage in excess of 4% at the base of the microstructure
gave rise to displacement of solvent from the microstructure
due to shrinkage with a velocity in excess of 0.01mm/s, which

demonstrates the rapid release of materials from a polymer
microstructure gel as a result of sudden change in surface
properties.

[0071]   There are numerous potential applications for
arrays of microstructures to which a high density of reactive
monomer or heteropolymer species have been attached. One
example is to use patterned synthesis of different
oligonucleotides on the array elements and hybridize labeled
DNA or RNA to this array, resulting in a very sensitive
version of a "DNA chip". This would allow one to detect·
expression at lower levels than is possible with chips that
utilize monolayers of DNA or to use much less expensive
equipment to read the array.

[0072]   The concept could be further generalized by making
arrays of DNA, RNA, peptide or other heteropolymer in high
density on microstructures in a patterned fashion such that
these arrays contain a selection of heteropolymers in known
positions that bind specific target molecules or classes of
target molecules that can be used in diagnostic tests.
Because of the high density of heteropolymers on the arrays,
the diagnostic detection limits will be much more sensitive,
and/or the equipment required to read the array will be
simpler and cheaper.

[0073]   Another example again involves making an array of
DNA oligonucleotides but to specifically make a set of
oligonucleotides that upon release from the microstructures
and hybridization (self assembly) followed by extension,
ligation and polymerase chain reaction (methods well known in
the art) could be used to rapidly synthesize large pieces of
double stranded or single stranded DNA.

[0074]   Another example involves making an array of DNA,
RNA, peptide or other heteropolymer in high density on
microstructures in a patterned fashion in such a way that

each of the elements (or each group of elements) in the array have a different heteropolymer structure attached. The array could be used as a library in a molecular evolution approach in which the individual array elements are assayed for some function (for example, binding, catalytic activity, signal transduction), the best ones are selected, and the heteropolymers selected from the first round of screening for activity are used, potentially along with computer modeling, to generate a new library. The library could be made as before and screened and the process continued until the desired function is optimized.

[0075]   While one or more embodiments of the present invention have been illustrated in detail, the skilled artisan will appreciate that modifications and adaptations to those embodiments may be made without departing from the scope of the present invention as set forth in the following claims.